



Optimizing the Effect of Aloe Vera Gel, Gum Arabic, and Liquid Smoke Coatings on Microbial Changes of Lactic Cheese

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ARTICLE INFO	ABSTRACT
Article type: Research Paper	Introduction: Cheese, as a widely consumed food product, is highly susceptible to spoilage by microorganisms such as fungi and bacteria during storage. This study aimed to investigate the effectiveness of an edible coating formulated with liquid smoke essence (0, 0.5 and 1% v/v), aloe vera gel (0, 10 and 20% v/v) and gum Arabic (0, 0.5 and 10% w/v) on controlling microbial growth in lactic cheese. Additionally, the process was optimized using the response surface methodology.
Article History: Received: 22 Oct 2024 Accepted: 22 Feb 2025	Methods: Lactic cheese was produced and cut into 3×3×3 cm. The cheese samples were immersed in the coating solutions for 5 minutes and then incubated at 8°C with 85% relative humidity for 8 hours to allow drying. The coated samples were packaged and stored at 4°C for 21 days. Microbial analysis was conducted throughout the storage period, including total bacterial count and mold and yeast enumeration.
Keywords: Coating Lactic cheese Microbial spoilage Optimization	Results: The results demonstrated that the lowest bacterial count (4.278 Log CFU/g) was observed in samples coated with the highest concentrations of liquid smoke essence and aloe vera gel. Although gum Arabic contributed to reducing the total bacterial count, its effect was less pronounced than the other treatments. Regarding mold and yeast growth, the best results (2.477 Log CFU/g) were obtained with the highest level of liquid smoke essence, while other parameters did not show a significant impact.
	Conclusion: According to the optimization model, the most effective coating formulation for maximizing cheese shelf life, consists of 1% v/v liquid smoke essence, 20% v/v aloe vera gel and 10% w/v gum Arabic.

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Introduction

Cheese is one of the most widely consumed dairy products. It is rich in proteins, essential nutrients, minerals, short-chain unsaturated fats, and some trans-unsaturated fats. Its high fat and protein content make it an energy-dense and nutritionally valuable food. Recent advancements in nutritional science have further emphasized the beneficial role of cheese in human health and nutrition [1].

Lactic cheese falls into the category of fresh (un-ripened) and semi-hard cheeses, produced through the coagulation of fresh milk, followed by heating after a reduction in pH. This cheese is ready for consumption immediately after production and is characterized by a white or

creamy-white color, along with a distinctive natural aroma and taste [2].

Due to its complex composition, cheese is highly susceptible to microbial contamination, particularly by fungi and bacteria, which can significantly compromise its quality [3]. Among the most critical pathogens associated with cheese contamination are *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and enteropathogenic *Escherichia coli* [4].

The most common molds found in cheese belong to the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Monilia*, and *Alternaria*. Among these, *Aspergillus* and *Penicillium* are the most prevalent. Due to the anaerobic conditions created during cheese production, mold growth

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is typically restricted to the surface. Consequently, numerous studies have focused on controlling microbial contamination in cheese [5, 6].

Coating effectively extends shelf life, regulates physicochemical changes, and minimizes contamination [7]. Since food products are highly susceptible to microbial degradation, selecting appropriate packaging methods is crucial for preserving food quality and safety. Edible films and coatings create a semi-permeable barrier around the product, slowing down quality deterioration, extending shelf life, delaying surface dehydration, preventing gas exchange, and inhibiting undesirable color changes [8]. These coatings are typically applied through dipping, spraying, or brushing [9].

Khodayi Kalaleh et al. (2021) reported that during a 30-day storage period of lactic cheese, the lowest microbial count was observed in samples coated with basil seed mucilage combined with the highest concentration of black cumin extract [10]. Similarly, Notaq et al. (2019) found that the antimicrobial and antifungal properties of chitosan, along with the potent fungicidal effects of natamycin, effectively inhibited the growth of most microbial species in Iranian ultrafiltered cheese coated with these compounds [11]. Mousavi et al. (2020) also demonstrated that a reduction in the growth of psychrophilic and thermophilic bacteria, as well as mold and yeast, was attributed to the application of an edible coating formulation containing sodium alginate, carboxymethyl cellulose, and garlic extract over a 21-day storage period [12].

In recent years, gums have gained significant attention in developing edible coatings. Intermolecular interactions primarily govern their film-forming properties, including mutual, electrostatic, hydrophobic, ionic and inter/intramolecular forces [13].

Gum Arabic, also known as acacia gum, is an edible hydrocolloid derived from *Acacia senegal* and *Acacia seyal* plants [14]. It is widely used in the pharmaceutical and food industries as an emulsifier and stabilizer in various products, including tablets, puddings, fillers, candies, and chewing gum [15].

Aloe vera gel is a natural, transparent, odorless, and environmentally friendly substance that easily dissolves in water. The presence of saponins and salicylic acid known for their

antifungal properties can effectively inhibit the growth and proliferation of fungi [16].

For centuries, smoking has been used as a traditional method for preserving food, particularly meat [17]. In modern applications, liquid smoke, produced by condensing wood smoke generated through the pyrolysis of sawdust or wood chips and subsequently removing carcinogenic polycyclic aromatic hydrocarbons is widely utilized [18]. The antimicrobial properties of commercial liquid smoke may vary depending on its source composition and the extraction and concentration methods used. Among the key bioactive components of smoke, phenolic compounds, carbonyls, and organic acids contribute to its characteristic taste, color, antioxidant activity, and antimicrobial effects [19]. Studies have demonstrated that smoke's antimicrobial and antioxidant properties are primarily linked to its phenol content. Phenols and their derivatives exert antimicrobial effects by denaturing bacterial proteins and disrupting the cell membrane, ultimately inhibiting bacterial growth [20].

The antimicrobial effectiveness of carbonyl compounds in smoke can be attributed to the presence of various aldehydes and ketones. Carbonyls inhibit microbial growth by penetrating the cell wall and deactivating enzymes within the cytoplasm and cell membrane. Even if carbonyls do not fully penetrate microbial cells, they can suppress growth by interfering with nutrient absorption [21].

Acetic acid exerts its antimicrobial effect by lowering pH, which leads to enzyme denaturation and increased cell membrane permeability ultimately inhibiting bacterial growth. The antimicrobial efficacy of acetic acid varies depending on its concentration, pH, environmental conditions, temperature, and the species or strain of bacteria present [22].

Optimization is improving a system, process, or production method to maximize efficiency and benefits. Response surface methodology (RSM) is a statistical approach to model and analyze data in which multiple variables influence a desired response. The primary objective of applying this method is to determine the optimal set of operating conditions to achieve specific target characteristics [23].

This study aimed to develop an edible coating formulated with liquid smoke essence, aloe vera gel, and gum Arabic to control microbial growth in lactic cheese and identify the effective coating formulation over a 21-day storage period.

Material and Methods

For microbial analysis, plate count agar and YGC agar media were purchased from Pars Nano Chemistry Company (Iran). Food-grade gum Arabic was obtained from Arvin Chemical Company (Iran) and liquid smoke essence was sourced from Niko Chemical Company (Iran). Aloe vera gel was procured from the local market.

The equipment used in this study included an ultrasonic homogenizer (UPS 1200, Nasir Research Iranian Technology Company, Iran), a laboratory scale with 0.001 precision (TA 313S, Sartorius, Germany), a refrigerated incubator (IPP 55, Memmert, Germany), a standard incubator (IN55, Memmert, Germany), an oven (UFA 500, Memmert, Germany), a pH meter (PB11, Sartorius, Germany), an electromagnetic heater (MS-HS-, Dragon Lab, Germany), a vortex mixer (MX-F, Dragon Lab, Germany), and Whatman filter paper (Pakistan). Additionally, disposable plates, samplers, and glass containers were utilized.

Producing Lactic Cheese

Raw milk was heated for pasteurization. Then, 2.4% v/v of a 20% w/v lactic acid solution was added and stirred for approximately 20 seconds [10]. Finally, curds were drained, pressed, and cut into 3×3×3 cm cubes.

Preparation of Coating Solutions

Gum Arabic solutions (5% and 10% w/v) were prepared at 40°C, while aloe vera gel solutions (10% and 20% v/v) were prepared after pasteurization at 75°C for 5 minutes [24, 16].

The prepared solutions were combined, and liquid smoke essence (0.5% and 1% v/v) was added. The mixture was homogenized using an ultrasonic homogenizer. Based on the experimental design generated by the software, a total of 18 solutions with different material ratios were formulated.

Coating of the Samples

The cheese samples were immersed in the prepared coating solutions, and any excess solution was removed from their surface. The coated samples were incubated at 8°C with 85%

relative humidity for 8 hours. Finally, they were packaged and stored at 4°C for 21 days [10].

Bacterial Total Count

The cheese samples were immersed in the prepared coating solutions, and any excess solution was removed from their surface. The coated samples were incubated at 8°C with 85% relative humidity for 8 hours. Finally, they were packaged and stored at 4°C for 21 days [10].

Mold and Yeast Count

To enumerate mold and yeast, 0.1 mL of each dilution was spread onto the surface of YGC agar (Yeast Glucose Chloramphenicol Agar) and incubated at 25°C for 5 days. Subsequently, yeast and mold colonies were counted [12].

Statistical Analysis

This study applied the response surface methodology (RSM) using Design-Expert software version 11.03. The selected experimental design was a central composite, face-centered model (α_1) with four central points. The independent variables included gum Arabic, aloe vera gel, and liquid smoke essence, while the dependent variables were total bacterial count and mold and yeast counts. Based on this design, 18 experimental runs were generated. In the final models, X_1 represented aloe vera gel (0, 10, and 20% v/v), X_2 represented gum Arabic (0, 5, and 10% w/v), and X_3 represented liquid smoke essence (0, 0.5, and 1% v/v). The significance of the model coefficients was determined by P-values, where values greater than 0.05 were considered non-significant and subsequently removed from the models. The fitted models were evaluated using the correlation coefficient (R^2) and adjusted correlation coefficient (R^2_{adj}). Additionally, 3D surface plots were utilized to visually and graphically assess the effects of the independent variables on the response variables [25].

Results and Discussions

Microbial tests were conducted after a 21-day storage period, and the results are presented in Table 1. Additionally, an analysis of variance (ANOVA) table was used to assess the significance of the model parameters (Table 2). As shown in Table 2, the proposed model for total bacterial count followed a quadratic form, with all three independent variables (aloe vera gel, gum Arabic, and liquid smoke essence) exhibiting significant effects in their linear.

Furthermore, the interaction between aloe vera gel and liquid smoke essence and their quadratic terms were also statistically significant ($p < 0.05$).

The fitted model followed a linear form for mold and yeast count, in which only liquid smoke essence had a significant effect ($p < 0.05$). At the

same time, other factors did not demonstrate statistical significance.

In each graph, the effect of two independent variables on a dependent variable was illustrated, while the third independent variable was held constant at its central value [26].

Table 1. Values of microbial parameters

Sample number	Bacterial total count (Log CFU/g)	Mold and yeast count (Log CFU/g)
1	4.579	3.380
2	4.653	3.505
3	4.977	3.812
4	4.556	2.612
5	4.924	3
6	4.672	3.778
7	4.568	3.397
8	4.544	3.740
9	4.886	3.778
10	4.579	3.380
11	4.278	2.477
12	4.544	3.322
13	5.176	3.875
14	4.908	3.778
15	4.568	3.397
16	4.342	3.653
17	4.929	3.826
18	4.954	2.698

Table 2. Analysis of variance of microbial qualitative responses

Variables	Bacterial total count (Log CFU/g)			Mold and yeast count (Log CFU/g)		
	Regression coefficients	F-value	P-value	Regression coefficients	F-value	P-value
X ₀	4.56			3.41		
linear						
X ₁	-0.1960	52.34	<0.0001	-	-	-
X ₂	-0.0647	5.70	0.0360	-	-	-
X ₃	-0.1384	26.10	0.0003	-0.4542	26.13	0.0001
two variables						
X ₁ X ₂	-	-	-	-	-	-
X ₁ X ₃	-0.0897	8.78	0.0129	-	-	-
X ₂ X ₃	-	-	-	-	-	-
grade two						
X ₁ ²	0.1342	7.60	0.0186	-	-	-
X ₂ ²	-	-	-	-	-	-
X ₃ ²	0.1232	6.41	0.0279	-	-	-
R ²	0.9185			0.6202		

Bacterial total count variations are presented in Figure 1. The highest concentrations of liquid smoke essence, aloe vera gel, and gum Arabic resulted in the lowest bacterial growth, reducing bacterial count by 18% compared to the control sample ($p < 0.05$) in the coated cheese samples. Figure 1-A illustrates that the highest bacterial total count was observed in samples coated with solutions containing the lowest aloe vera gel and liquid smoke essence concentrations. As the concentrations of smoke essence and aloe vera

gel increased, a corresponding decrease in bacterial total count was noted.

Similarly, Figure 1-B demonstrates that the highest bacterial total count occurred in samples coated with solutions containing the lowest aloe vera gel and gum Arabic percentages.

Figure 1-C highlights the significant effect of smoke essence on bacterial total count ($p < 0.05$) showing that samples with the lowest concentration of smoke essence had the highest bacterial growth. Although gum Arabic also

reduced bacterial total count, its effect was less pronounced than smoke essences. Overall, samples coated with solutions containing the minimum concentrations of smoke essence, aloe vera gel and gum Arabic exhibited the highest bacterial total count. The antimicrobial activity of liquid smoke essence can be attributed to the presence of phenolic compounds, aldehydes, and organic acids, such as formic acid and acetic acid, which influence the membrane permeability of microorganisms, particularly Gram-positive bacteria [27]. Similarly, anthraquinones and saponins in aloe vera gel contribute to its antibacterial properties against a wide range of Gram-positive and Gram-negative bacteria [15]. In the case of gum Arabic, its antimicrobial effects can be associated with its high terpene content [28, 29].

Findings from previous studies also support the positive effects of coatings and essential oils in controlling microbial growth. Ali Mohammadzadeh et al. (2020) and Bakshi et al. (2020) reported similar results when investigating the coating of Iranian lactic cheese and white cheese with alcoholic extracts of black cumin and basil, respectively, which effectively inhibited the growth of mesophilic bacteria [29, 30]. Additionally, Destevia et al. (2023) examined the antimicrobial activity of liquid smoke derived from cocoa pod shells against Gram-negative bacteria (*Escherichia coli*, *Salmonella choleraesuis*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), reporting that higher smoke concentrations enhanced the antimicrobial efficacy of coatings [31].

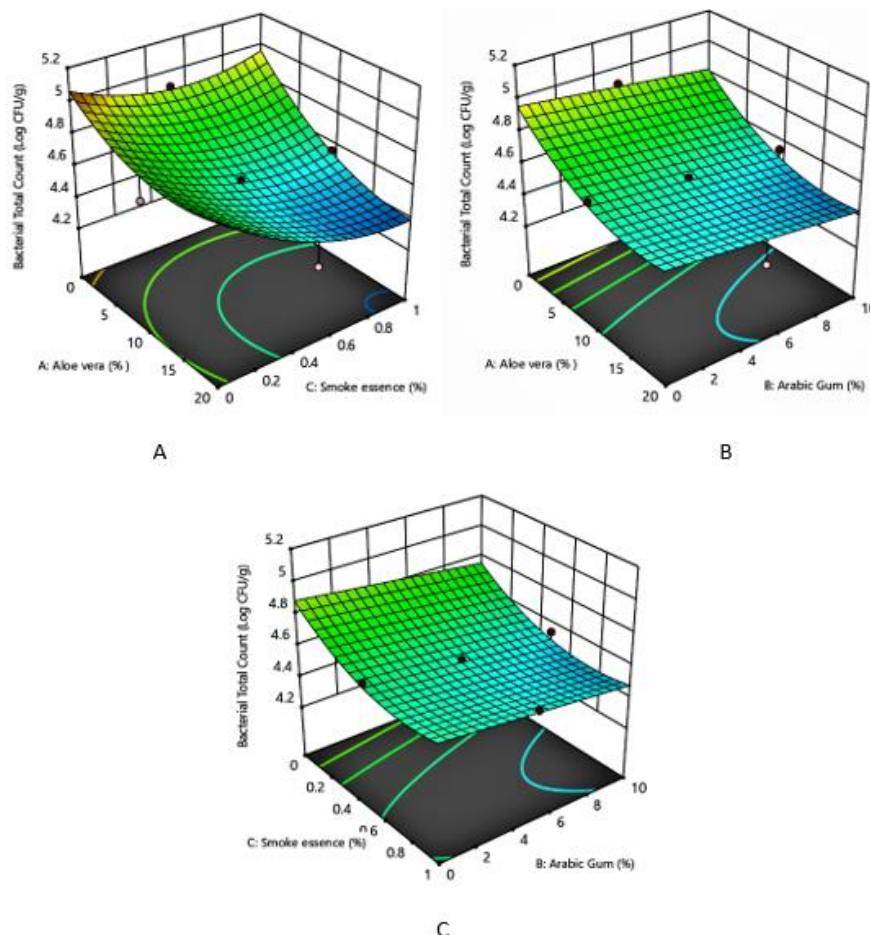


Figure 1. (A): 3D image of the effect of aloe vera gel and smoke essence on bacterial total count; (B): 3D image of the effect of aloe vera gel and gum Arabic on bacterial total count; (C): 3D image of the effect of gum Arabic and smoke essence bacterial total count.

Regarding mold and yeast counts, the results indicated that only the linear term of smoke essence had a significant effect ($p < 0.05$). Figure 2 presents the 3D response surface plots corresponding to these findings. The results demonstrated that by incorporating 1% v/v, smoke essence, 20% v/v aloe vera gel and 10% w/v gum Arabic, the growth of mold and yeast reduced by 36% compared to the control sample. Figures 2-A, B and C illustrate that the lowest mold and yeast counts were observed in samples containing the highest concentration of smoke essence. However, aloe vera gel and gum Arabic did not exhibit a significant effect.

Molds contribute to cheese spoilage through the action of their lipases and proteases. Also, some molds can produce toxic secondary metabolites, such as mycotoxins and cause an unpleasant taste and odor. The predominant mold species isolated from cheese is *Penicillium* [32]. The application of smoke essence resulted in a significant reduction in mold and yeast counts.

The coatings containing the highest concentration of smoke essence demonstrated the most crucial protective properties.

The difference in the effect of gum Arabic and aloe vera gel on bacterial populations compared to molds and yeasts may be attributed to their ability to reduce free water due to their film-forming properties. Since bacteria generally require higher water activity than molds and yeasts, this property may explain their more pronounced effect on bacterial reduction than mold and yeast populations [33].

In summary, the results of this study indicate that smoke essence had a highly significant impact ($p < 0.05$), playing a major role in reducing microbial growth over 21 days of refrigerated storage. Furthermore, coating cheese with biopolymers such as aloe vera gel and gum Arabic significantly controlled the growth of aerobic mesophilic bacteria, primarily by limiting oxygen transfer and availability [34].

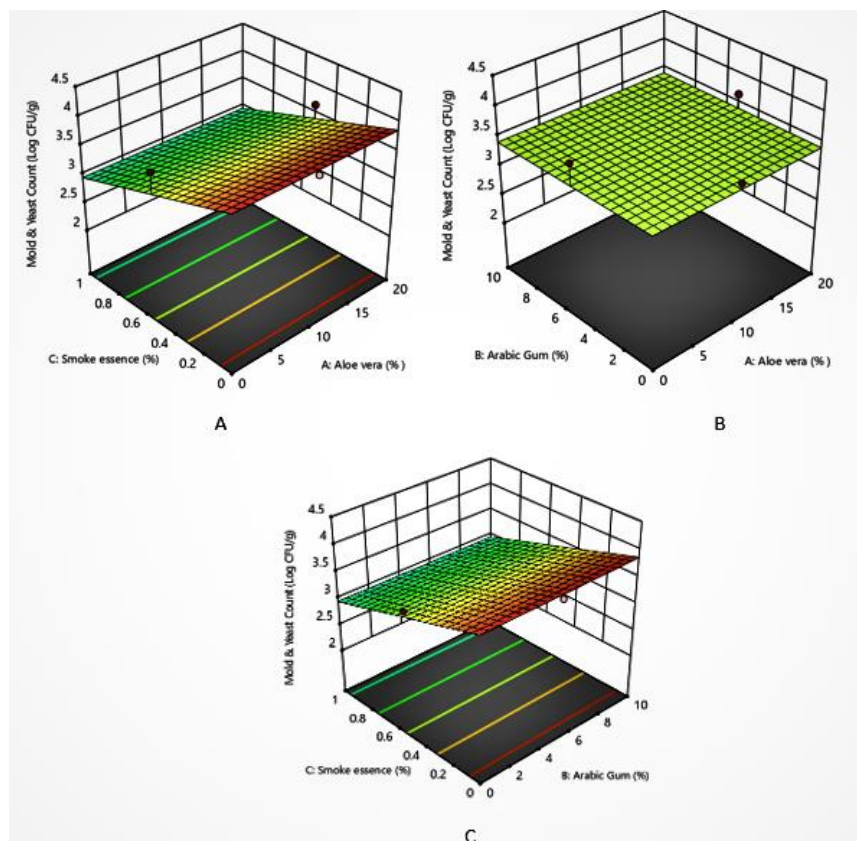


Figure 2. (A): 3D image of the effect of aloe vera gel and smoke essence on mold and yeast count; (B): 3D image of the effect of aloe vera gel and gum Arabic on mold and yeast count; (C): 3D image of the effect of gum Arabic and smoke essence on mold and yeast count.

The findings of previous studies align with those of the present investigation. Notaq et al. (2019) coated Iranian ultra-filtered cheese with chitosan and natamycin, reporting that their antifungal properties effectively reduced mold growth in the samples [10]. Similarly, Sajjadi et al. (2016) examined the effect of Aloe vera gel on mold populations in ultra-refined white cheese and reported comparable inhibitory effects on mold growth [34].

Optimizing

The primary objective of this research was to develop an optimal edible coating for preserving lactic cheese by utilizing three combinations of aloe vera gel, gum Arabic, and smoke essence.

The optimization process was conducted using Design Expert software to achieve this goal. A suitable range was defined for each independent variable, and specific weights were assigned to the dependent variables based on their relative importance. The software then optimized the values accordingly [35].

For maximum inhibitory effectiveness, the optimized formulation suggested by the software consisted of 1% v/v liquid smoke essence, 20% w/v aloe vera gel and 10% w/v gum Arabic.

Conclusion

The results of this study demonstrated that coating cheese samples, effectively controlled microbial populations by limiting oxygen exposure, regulating environmental conditions and leveraging the antimicrobial properties of coating materials such as smoke essence. The lowest microbial counts were observed in samples containing the highest concentration of smoke essence. In conclusion, applying an edible coating composed of liquid smoke essence, aloe vera gel and gum Arabic can potentially extend the shelf life of lactic cheese during refrigerated storage.

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Conflicts of Interest

Authors have no conflicts of interest.

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